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New Record of Two Species in Stichotrichous Ciliates (Ciliophora: Stichotrichia) from Korea

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ABSTRACT

Two stichotrichous ciliates, *Amphisiella annulata* (Kahl, 1928) and *Pseudoamphisiella alveolata* (Kahl, 1932), were collected from the Yellow Sea in Incheon, Korea and were identified as new to Korea. The main diagnostic key to the species of the genera are that the two species share the features of two macronuclear nodules, one marginal row per side, and transverse cirri. *A. annulata* is distinguishable from other members in the genus mainly due to the several ring-shaped (hollow) structures in its cytoplasm and its wide and narrowly arranged amphisiellid median cirral row. *P. alveolata* has a conspicuous alveolar layer and two conspicuous macronuclear nodules, but no frontoterminal cirri.

Key words: *Amphisiella annulata*, *Pseudoamphisiella alveolata*, marine ciliate, Korea

INTRODUCTION

Thus far, more than 170 ciliates have been recorded in South Korea (Shin and Kim, 1988; Yoo et al., 1988; Yoo and Kim, 1990; Shin, 1994; Lee and Kim, 2000; Lei et al., 2005a, b; Xu et al., 2006; Gong and Choi, 2007; Gong et al., 2007a, b; Xu and Lei, 2007). Eleven marine ciliates of the subclass Stichotrichia have been reported in Korea: *Holosticha hamulata*, *Holosticha heterofoissneri*, *Hemigastrostyla enigmatica*, *Metastrongylidium distichum*, *Metaurostylopsis marina*, *Metaurostylopsis salina*, *Metaurostylopsis songi*, *Oxytricha marina*, *Protogastrostyla pulchra*, *Tachysoma multinucleate*, and *Tunicothrix rostrata*. The subclass Stichotrichia has the following morphological features: a dorsoventrally compressed shape; cirri of the somatic ventral ciliature arranged in a longitudinal, sometimes spiraled manner; and paramembranelles of the adoral zone of oral polykinetids in the form of a "collar" (Lynn, 2008).

The present study describes two marine benthic stichotrichous ciliates that belong to the aforementioned genera: *Amphisiella* Gourret & Roeser, 1888 and *Pseudoamphisiella* Song, 1996. Both genera are new to Korea. Shin (1994) reported *A. acuta* previously in Korea. This species, however, was newly combined in *Paramphisiella* by Berger (2008).

A role of ciliate in microbial food web and the inhabitants of the benthic community are limited to planktonic ciliates (Dopheide et al., 2008). Although ciliates are ecologically important as integral components that drive nutrients to metazoans in aquatic ecosystems, little is known regarding their influence in Korea. To provide faunistic information and in turn application to other research fields as a basic resource, we investigated marine ciliates through live observation, protargol impregnation, and analyses of small subunit ribosomal DNA (SSU rDNA) sequences as a molecular characteristic.

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MATERIALS AND METHODS

Sample collection and identification

The specimens used in this study were collected from Incheon harbor (salinity, 29.6‰; temperature, 26.7°C; 37° 26′N, 126°35′E) in Incheon, Korea, from July to August of 2008 (Fig. 1). Glass slides were used as artificial substrates (Xu et al., 2009). The slides were kept in the sea for one week at a depth of approximately 1 m and were taken to the laboratory for the taxonomic assessment.

Cultures were maintained in both Petri dishes and 50 mL tissue culture flasks (Greiner Bio-one, Frickenhausen, Germany). Rice grains were used to enrich bacterial growth in the culture and the enriched bacteria were grazed by ciliates as a food resource. Living specimens were observed under a light microscope (Leica DM2500, Wetzlar, Germany) at 50 to 1,000 magnification. Protargol impregnation was carried out to reveal their infraciliatures (Foissner, 1991).

Terminology and classification were mainly followed according to Berger (2006) and Lynn (2008). The abbreviations indicated in Figs. 2 and 4 include the following: AZM, adoral zone of membranelle; PM, paroral membrane; EM, endoral membrane; FC, frontal cirrus; BC, buccal cirrus; FT, frontoterminal cirrus; TC, transverse cirrus; ACR, am-

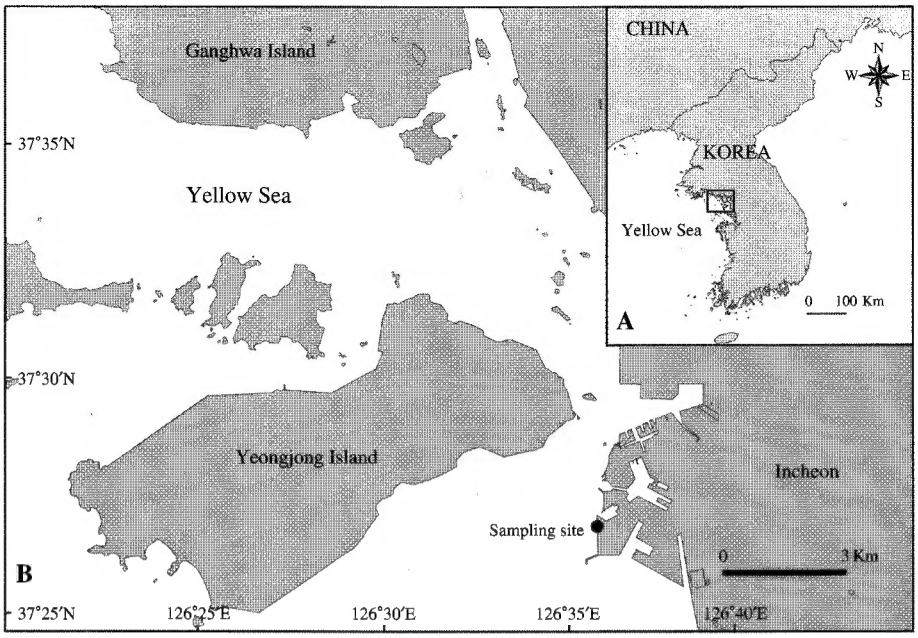

Fig. 1. Sampling locality. (A) Coast of Incheon; (B) enlarged sampling site, Incheon harbor.

phisiellid median row; VR, ventral row; LMR, left marginal cirral row; RMR, right marginal cirral row; CC, caudal cirrus; DK, dorsal kinety; Ma, macronuclear nodule; Mi, micronucleus; CV, contractile vacuole.

DNA sequence determination

Genomic DNA from a single specimen was extracted using a RED-Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, USA) according to the manufacturer's protocol. New EukA (5′-CTG GTT GAT YCT GCC AGT-3′), modified from Medlin et al. (1988) and LSU rev2 (Sonnenberg et al., 2007) primers were used for the PCR amplification of nearly complete SSU rDNA. The optimized conditions for this process were as follows: Denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 4 min, and then a final extension step at 72°C for 7 min. PCR products were purified with the QIAquick® PCR Purification Kit (QIAGEN, Chatsworth, USA). Three internal primers were newly designed based on the representative stichotrichs: 18S+810, 5′-GCC GGA ATA CAT TAG CAT GG-3′, 18S+1470, 5′-TCT GTG ATG CCC TTA GAT GTC-3′, and 18S-300, 5′-CAT GGT AGT CCA ATA CAC TAC-3′. Sequencing was carried out using an ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA). The sequencing fragments of SSU rDNA were combined via BioEdit (Hall, 1999) and were aligned according to Clustal X 1.81 (Jeanmougin et al., 1998). Genetic distances of each species were calculated using Mega 3.1 (Kumar et al., 2004) with the Kimura two-parameter distance method (Kimura, 1980).

SYSTEMATIC ACCOUNTS

Phylum Ciliophora Doflein, 1901
Class Spirotrichea Bütschli, 1889
Order Stichotrichida Fauré-Fremiet, 1961
Family Amphisiellidae Jankowski, 1979
Genus *Amphisiella* Gourret and Roeser, 1888

[1]**Amphisiella annulata* (Kahl, 1928) (Table 1, Figs. 2, 3)

Holosticha annulata Kahl, 1928, p. 212, Fig. 44f.
Amphisiella (*Holosticha*) *annulata* Kahl, 1928: Kahl, 1932, p. 590, Fig. 1121.
Amphisiella annulata (Kahl, 1928) Kahl, 1930-5: Carey, 1992, p. 179, Fig. 701.
Amphisiella annulata (Kahl, 1928) Borror, 1972: Berger, 2004, p. 1, Figs. 1-23; Hu et al., 2004, p. 363, Figs. 4, 5, 8.

Material examined. One population was obtained from the Incheon harbor on the 14th of August, 2008.
Description. Cell in life 75-145 × 20-45 μm, usually 119 ×

[1]*고리양열하모충(신칭)

Table 1. Morphometric characteristics of *Amphisiella annulata* (this study, first line) and other populations (Berger, 2004, second line; 1996 population from Hu et al., 2004, third line) from protargol-impregnated specimens.

	Min	Max	Mean	SD	SE	CV	n
Body length	118	219	158.9	25.31	5.97	15.9	18
	68	121	96.4	12.30	2.30	12.8	30
	102	203	167.3	24.69	6.37	14.8	15
Body width	26	64	44.5	11.05	2.26	24.8	24
	23	55	37.4	7.70	1.40	20.5	29
	45	80	59.3	10.60	2.74	17.9	15
Length of buccal field	42	74	60.1	9.47	2.12	15.8	20
	26	50	40.6	5.10	0.90	12.5	29
	48	78	65.7	8.63	2.23	13.1	15
Number of adoral membranelles	42	61	50.2	5.27	1.01	10.5	27
	31	57	47.2	5.90	1.10	12.4	28
	41	62	52.2	5.70	1.52	10.9	15
Number of frontal cirri	3	3	3	0	0	0	17
	3	4	3	0.2	0	6	30
	3	3	3	0	0	0	15
Number of cirri behind of the right frontal cirrus	1	1	1	0	0	0	21
	1	1	1	0	0	0	30
	1	1	1	0	0	0	15
Number of left buccal cirri	1	1	1	0	0	0	25
	1	1	1	0	0	0	30
	1	1	1	0	0	0	15
Number of cirri left of ACR	3	3	3	0	0	0	19
	3	4	3.1	0.30	0	8.3	30
	3	3	3	0	0	0	15
Number of cirri in ACR	38	59	49	5.50	1.42	11.2	15
	25	54	44.5	5.70	1.20	12.9	24
	45	61	49.9	13.04	5.83	26.2	15
Number of pretransverse ventral cirri	2	2	2	0	0	0	23
	2	2	2	0	0	0	28
	2	2	2	0	0	0	15
Number of transverse cirri	4	7	5.7	0.62	0.13	10.9	24
	5	6	6	0.20	0	3.1	29
	5	7	5.9	0.46	0.12	7.7	15
Number of left marginal cirri	37	51	42.1	3.57	0.87	8.5	17
	25	41	34.6	3.50	0.60	10.0	29
	36	46	40.9	3.50	0.93	8.5	15
Number of right marginal cirri	36	51	42.7	4.60	1.15	10.8	16
	28	41	34.1	3.20	0.60	9.3	27
	36	49	42.4	4.29	1.15	10.1	15
Number of dorsal kineties	7	9	7.7	0.78	0.22	10.6	12
	6	8	6.4	0.60	0.10	9.2	23
	8	10	8.2	0.53	0.13	6.5	15
Number of macronuclear nodules	2	2	2	0	0	0	31
	2	2	2	0	0	0	30
	2	2	2	0	0	0	15
Number of micronuclei	2	8	4.4	1.36	0.27	30.8	26
	3	8	5.6	1.30	0.20	23.5	30
	2	6	3.5	0.98	0.21	28.2	21

ACR, amphisiellid median cirral row; CV, coefficient of variation in %; Max, maximum; Mean, arithmetic means; Min, minimum; n, number of individuals examined; SD, standard deviation; SE, standard error of mean. All measurements are in micrometers.

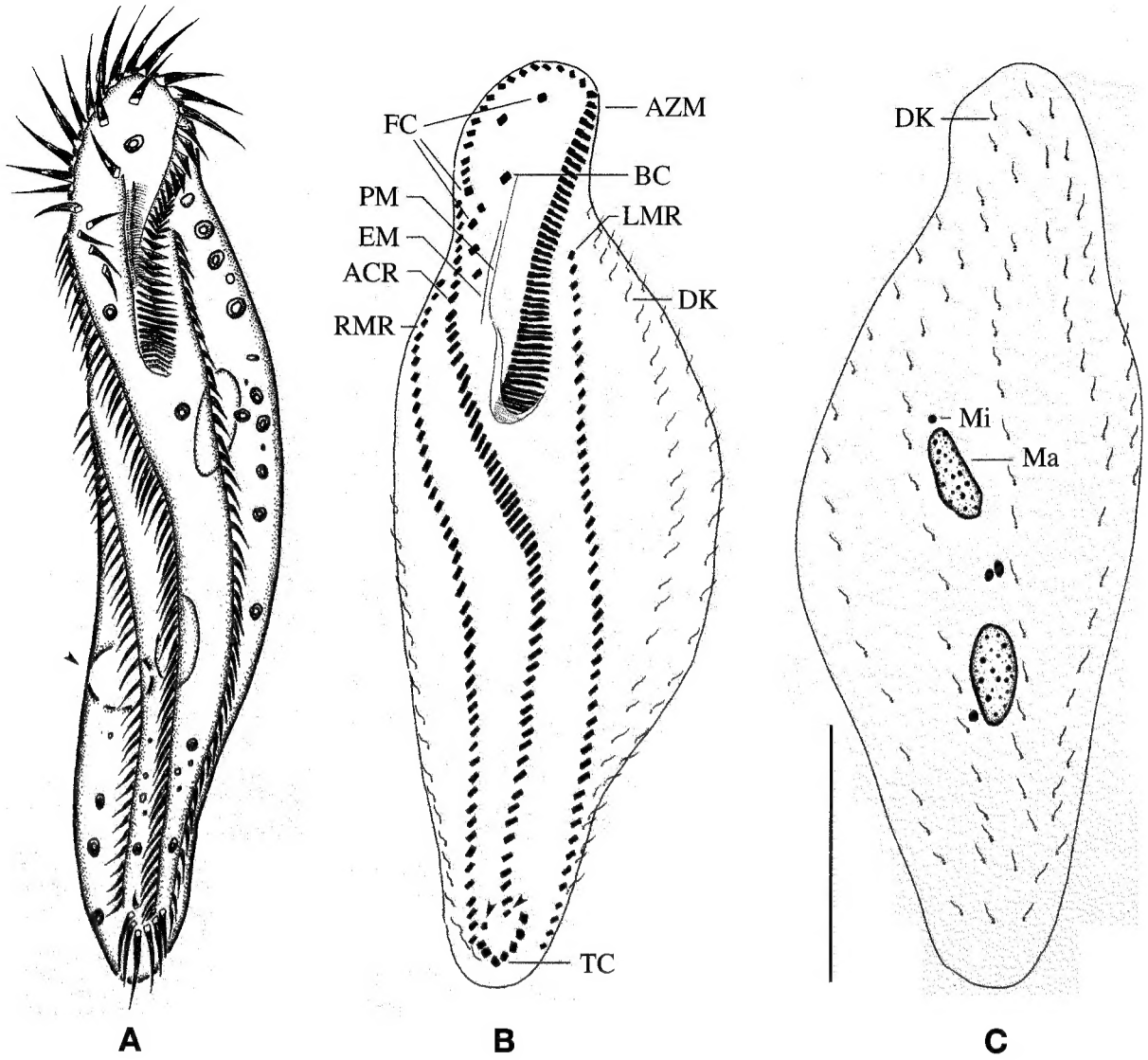

Fig. 2. Morphology and infraciliature of *Amphisiella annulata* from live (A) and after protargol impregnation (B, C). (A) Ventral view of live, arrowhead in (A) denote CV; (B, C) infraciliature of ventral (B) and dorsal (C) sides, arrowhead in (B) to show the pretransverse ventral cirri. Scale bar=50 μm.

35 μm. Body generally elongated elliptical; sigmoidal outline of body, convex at left middle portion (Fig. 2A); dorsoventrally flattened. CV located on right side of posterior third, about 7 μm in length (Figs. 2A, 3B). Ring-shaped (hollow sphere) structures scattered throughout the body, *ca* 2-4 μm in diameter (Fig. 3A, C, E). Colorless extrusomes around dorsal bristle arranged in lines along DK (Fig. 3F; *ca* 1 μm in diameter).

AZM distinct, about 1/3 of cell length, composed of about 50 membranelles; cilia of adoral membranelles *ca* 10 μm in length. Two elongated Ma positioned in center of body, 17-26 × 8-11 μm (Figs. 2C, 3I, K). Two to eight Mi, about 2-4 × 2 μm, scattered throughout the body (Fig. 2C). FC distinct; one of the three cirri, the rightmost frontal cirrus behind distal end of AZM (Fig. 3G). One BC, beside anterior end of PM (Fig. 3G). One cirrus behind right FC; three cirri left of ACR (Fig. 3K). ACR commencing near right frontal cirrus, narrowly arranged; the cirri widen in the middle part of body; cirri *ca* 5 μm in length. Left marginal cirri, 3-4 μm in length. RMR parallel to ACR, terminating near TC. TC, *ca* 8-13 μm in length. Two pretransverse cirri positioned ahead of TC (Fig. 3J, K). Seven to nine DK extending over the entire body with 2 to 3 kineties on lateral sides (Fig. 2C); dorsal cilia about 2 μm in length. CC absent.

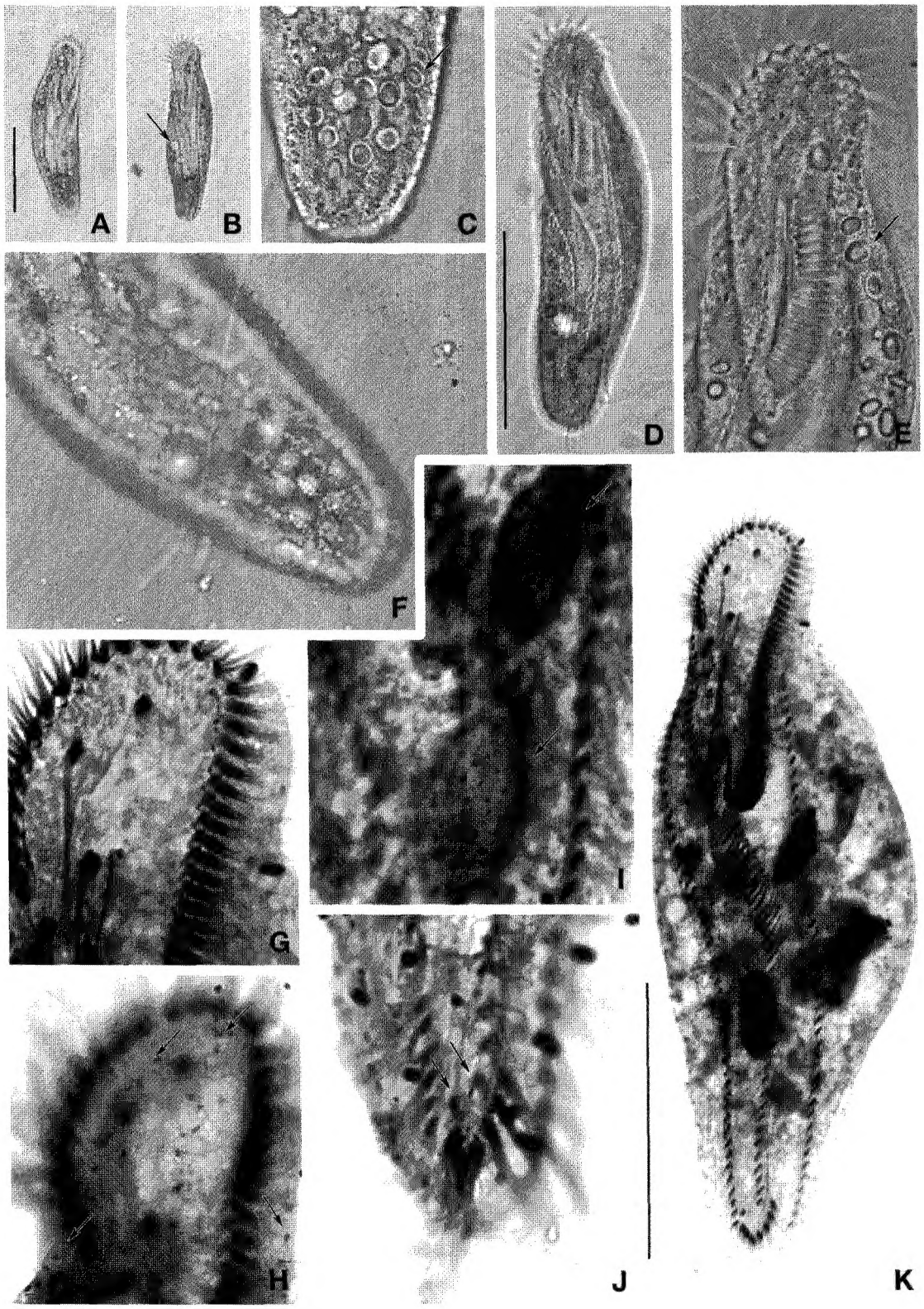

Fig. 3. Morphology and infraciliature of *Amphisiella annulata* from live (A-F) and after protargol impregnation (G-K). (B, D, E) Ventral and (A, C, F) dorsal views of live, (B) mark a contractile vacuole, arrow in (C, E) indicates ring-shaped hollow structures, (F) cortical granules around dorsal kinety; (G-K) infraciliature of ventral (G, I-K) and dorsal (H) sides, arrow in (H) shows the dorsal kineties, arrows in (I) indicate the macronucleus and denote pretransverse ventral cirri in (J, arrow). Scale bars=60 μm.

Table 2. Morphometric characteristics of *Pseudoamphisiella alveolata* (this study, first line) and another population (Song & Warren, 2000, second line) from protargol-impregnated specimens.

	Min	Max	Mean	SD	SE	CV	n
Body length	107.5	167.5	149	16.18	3.37	10.9	23
	75	159	96.6	24.48	5.77	25.3	18
Body width	70	105	87	10.15	2.27	11.7	20
	34	86	47.3	15.91	3.75	33.7	18
Length of buccal field	35	65	55.5	7.19	1.50	12.9	23
	24	42	35.1	5.63	1.33	16.1	18
Number of adoral membranelles	40	65	57.7	6.22	1.30	10.8	23
	47	59	50.9	3.72	1.24	7.3	9
Number of frontal cirri	3	3	3	0	0	0	20
	3	3	3	0	0	0	18
Number of buccal cirri	2	2	2	0	0	0	15
	2	2	2	0	0	0	18
Number of cirri in ventral row 1	11	16	13.7	1.23	0.27	9.0	21
	11	15	12.5	1.57	0.47	12.5	11
Number of cirri in ventral row 2	10	16	12.8	1.60	0.36	12.6	20
	10	14	12.2	1.47	0.44	12.1	11
Number of left marginal cirri	18	29	24.7	2.99	0.65	12.1	21
	14	20	17.0	1.83	0.56	10.9	11
Number of right marginal cirri	14	17	15.1	0.92	0.20	6.1	21
	12	14	13.1	0.83	0.25	6.3	11
Number of extra right marginal cirri	2	5	3.3	0.90	0.20	27.5	21
	–	–	–	–	–	–	–
Number of transverse cirri	10	18	15.4	1.62	0.34	10.5	23
	12	16	14.2	1.56	0.39	11.0	16
Number of caudal cirri[a]	10	15	12.6	1.22	0.26	9.6	22
	11	16	13.9	1.90	0.63	13.7	9
Number of dorsal kineties	9	13	11.1	1.45	0.44	13.0	11
	10	12	11.2	0.75	0.19	6.7	16
Number of macronuclear nodules	2	2	2	0	0	0	22
	2	2	2	0	0	0	16
Number of micronuclei	2	7	4.3	1.13	0.24	26.2	22
	2	5	3.5	1.21	0.37	35.1	11

[a], population from Song & Warren (2000); they did not separate it from extra right marginal cirri; CV, coefficient of variation in %; Max, maximum; Mean, arithmetic means; Min, minimum; n, number of individuals examined; SD, standard deviation; SE, standard error of mean. All measurements are in micrometers.

Distribution. Germany, Italy, China, and Korea (this study).

Remarks. Recently *Amphisiella* was redefined by Berger (2008) by the following features: (i) distinct TC; (ii) absence of CC; (iii) seawater habitat. In this genus, five species are included: *A. capitata* (Pereyaslawzewa, 1886), *A. annulata* (Kahl, 1928), *A. milnei* (Kahl, 1932), *A. turanica* Alekperov and Asadullayeva, 1999, and *A. ovalis* Fernandez-Leborans and Novillo, 1992.

A. annulata is characterized primarily by two Ma and numerous ring-shaped structures scattered throughout the body. *A. capitata* and *A. milnei* also have two Ma. These two species, however, can be easily distinguished from *A. annulata* by the number of ring-shaped structures in the cytoplasm. Although this structure can be absent in some populations of *A. annulata*, different cirral pattern or granule colors validate species identification from the above two species (Kahl, 1932; Berger, 2004; Hu et al., 2004). *A. capitata* has (i) no ring-shaped structures (*vs.* presence); (ii) only two cirri on the left ACR (*vs.* 3); (iii) a shorter and ordinary size of the ACR (*vs.* longer and widened in the middle of the body) (Berger, 2008). *A. milnei* has (i) only a few (less than two) ring-shaped structures (*vs.* several); (ii) yellowish cortical granules (*vs.* colorless); (iii) a different cirral pattern in the frontal area; (iv) an ACR of an ordinary size (*vs.* widen in

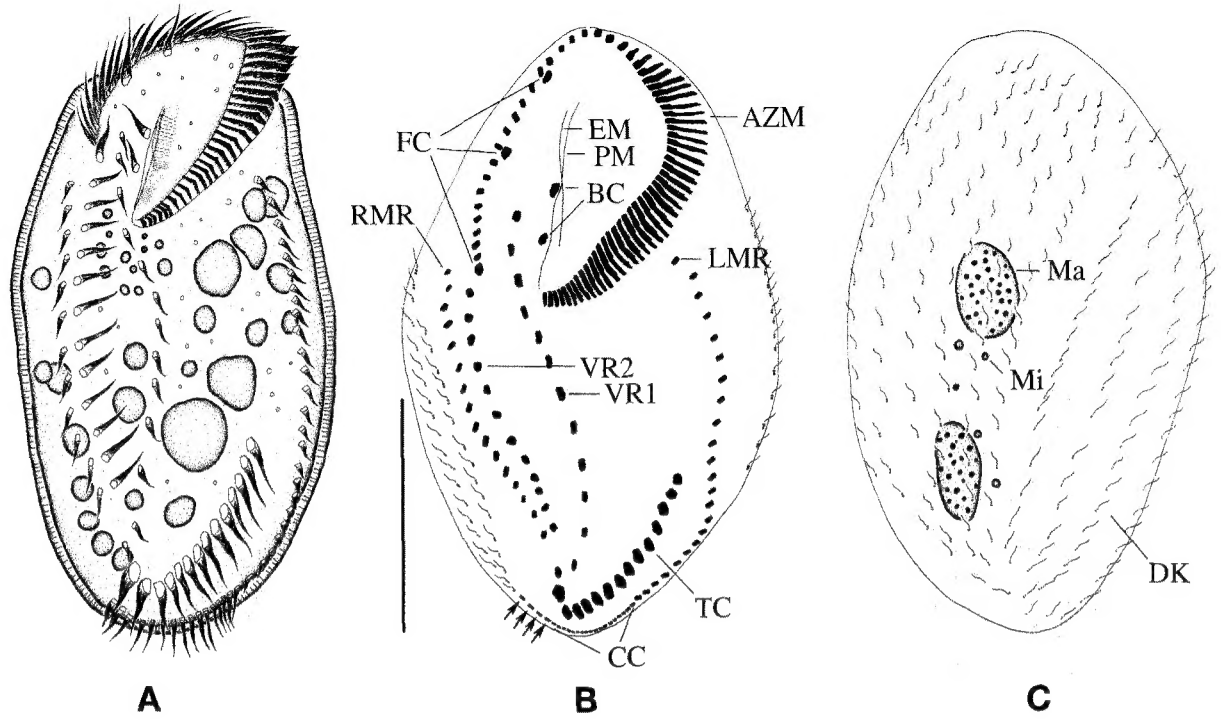

Fig. 4. Morphology and infraciliature of *Pseudoamphisiella alveolata* from live (A) and after protargol impregnation (B, C). (A) Ventral view of live; (B, C) infraciliature of ventral (B) and dorsal (C) sides, arrows in (B) to show extra right marginal cirri. Scale bar=60 μm.

middle of body) (Kahl, 1932; Berger, 2008). *A. turanica* and *A. ovalis* are rather conspicuous because they have more than two Ma, four and 32-45, respectively (Alekperov and Asadullayeva, 1999; Berger, 2008).

The present *A. annulata* population corresponds well with the previous descriptions by Kahl (1928, 1932). A morphometric comparison with previously described *A. annulata* populations (Italy and China) is shown in Table 1. Hu et al. (2004) described two populations of *A. annulata* (1996, 2000 in China). Both of these populations shared very similar morphometric data. The morphometric data of the Chinese *A. annulata* population (1996, China) were comparatively quite similar to those of the Korean population. The Italian population has a smaller body (96.4 *vs.* 158.9 μm in length) and fewer cirri (44.5 *vs.* 49 in ACR; 34.6 *vs.* 42.1 in LMR; 34.1 *vs.* 42.7 in RMR) than the Korean population. Although the ring-shaped structures and CV can be absent, this population showed conspicuous features (Berger, 2004; Hu et al., 2004).

The sequence length of the SSU rDNA in the Korean population is 1,669 bp (GenBank Accession number GU170843) with intra-specific variation revealing 1.5% relative to known *A. annulata* (DQ832260).

Order Urostylida Jankowski, 1979
Family Urostylidae Bütschli, 1889
[1]*Genus *Pseudoamphisiella* Song, 1996

[2]****Pseudoamphisiella alveolata*** **(Kahl, 1932)**
(Table 2, Figs. 4, 5)

Holosticha alveolata Kahl, 1932, p. 581, fig. 107.
Holosticha alviolata Kahl, 1930-5: Carey, 1992, p. 181, fig. 710.
Pseudoamphisiella alveolata: Song and Warren, 2000, p. 453, figs. 1-17; Hu and Suzuki, 2006, p. 44, figs. 1-49; Shao et al., 2006, p. 389, figs. 1-45.

Material examined. One population was found from the Incheon harbor, 24 July 2008.

Description. Size in life 100-213 × 50-78 μm, usually 135 × 67 μm. Body generally elongated and contractile, thus variable in shape and size, extended shape in motion resembling a sigmoid; contracted oval shape (Fig. 4A-C); dorsoventrally flattened.

Cell surface covered by conspicuous alveolar layer and small extrusomes; alveolar layer, 3-4 μm in depth; bar-like extrusomes sparsely arranged in a layer, *ca* 1.5 × 0.5 μm

[1]*위양열하모충속(신칭), [2]*유막위양열하모충(신칭)

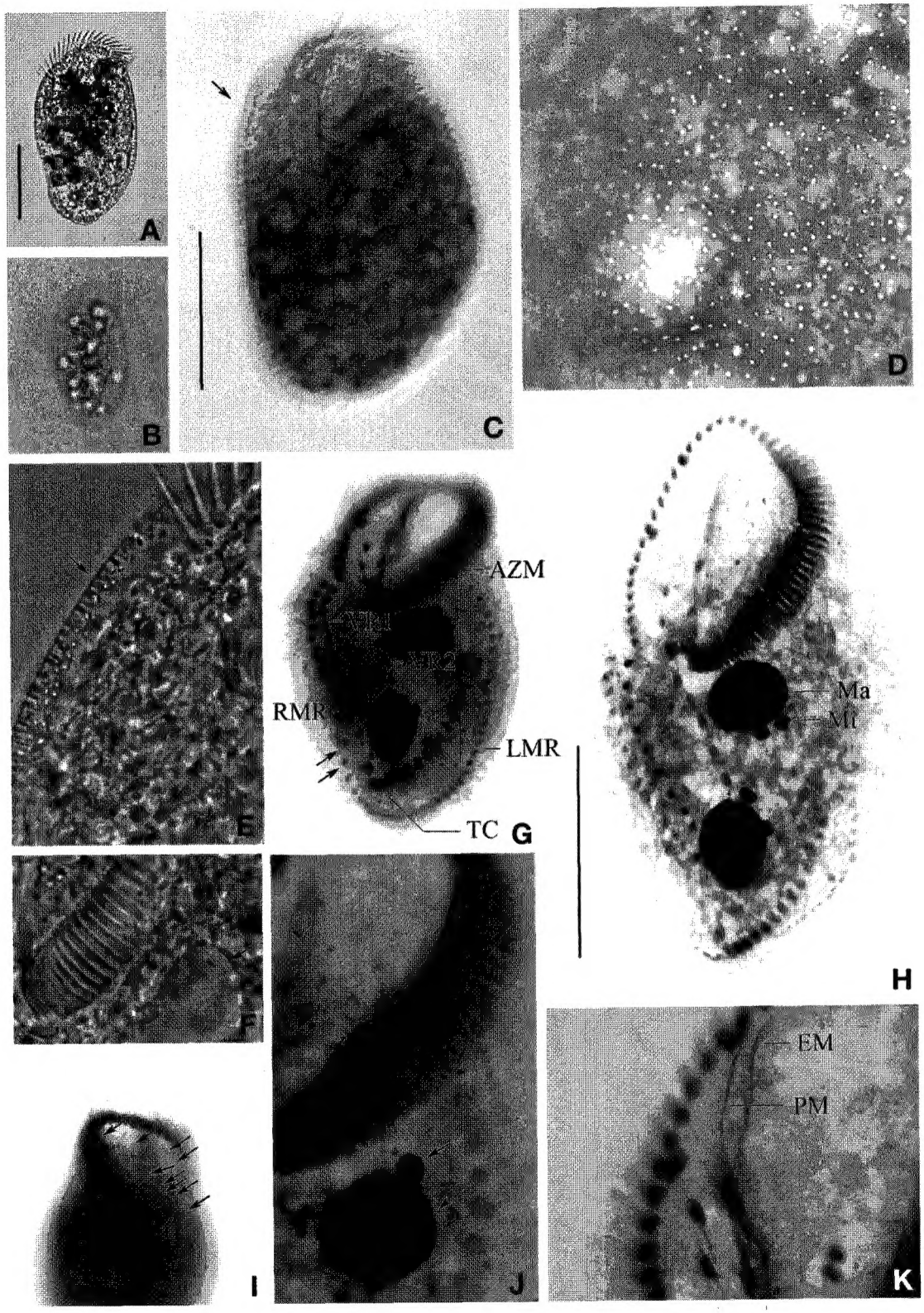

Fig. 5. Morphology and infraciliature of *Pseudoamphisiella alveolata* from live (A-F) and after protargol impregnation (G-K). (A, C, E, F) Ventral view, (B, D) dorsal view of live, (C, E) mark the alveoli layer and extrusome, arrow in (F) indicates macronucleus; (G-K) infraciliature of ventral (G, H, J, K) and dorsal (I) sides, arrows in (G) to show extra right marginal cirri, arrows in (I) indicate the dorsal kineties and denote the macronucleus (arrowhead) and micronucleus (arrow) in (J). Scale bars=60 μm.

(Figs. 4A, 5A, C-E, arrow); extrusome-linkage forming a cell surface polygon (Fig. 5D). Neither CV nor food vacuoles are observed in the cytoplasm.

AZM distinct, about 1/3 of the cell length, composed of about 58 membranelles (Figs. 4A, B, 5C, G, H); cilia of adoral membranelles short, *ca* 7-10 μm in length. Two elongated Ma positioned in center of body, 19-32 × 14-21 μm (Figs. 4C, 5G, H). Two to seven Mi, about 4 μm in diameter, scattered throughout the body (Fig. 5H, J). Three FC distinct, located near the AZM; one posterior cirrus of the three FC was close to the distal end (Fig. 4B). Two BC were separated by crossing EM (Fig. 5G, K). VR1 parallel to RMR, lying horizontally derived from the ventral groove; anterior VR1 close to the right-most FC; cirri of VR1, *ca* 6-8 μm in length (Fig. 5G). LMR, CC, and extra marginal cirri are arranged in the same line, which makes it difficult to distinguish one from the other (Fig. 4B, arrows, extra marginal cirri). TC highly developed and arranged in a J-shaped row. Anterior DK curved and expended to the posterior end (Fig. 4C); two to three kineties on the lateral side (Fig. 4B); dorsal cilia about 3 μm in length.

Distribution. Germany, China, Japan, and Korea (this study).

Remarks. *Pseudoamphisiella* Song, 1996 has a unique cirral pattern: the origin of the right marginal cirri and the absence of FT distinguish it from other hypotrichs (Song, 1996; Song et al., 1997). As a type species of *Pseudoamphisiella*, *Holosticha lacazei* (Maupas, 1883) was newly combined in this genus by Song (1996). In addation, Song et al. (1997) suggested a new family, Pseudoamphisiellidae. Subsequently, *P. alveolata* (Kahl, 1932) and *P. quadrinucleata* Shen et al., 2008 were recorded, and these three species are known to be in this genus at present (Song and Warren, 2000; Shen et al., 2008). Although Berger (2006) and Lynn (2008) assigned this genus to Urostylidae, systematic positioning based on molecular analyses showed that Pseudoamphisiellidae clustered away from Urostylidae. The issue, therefore remains unclear (Shao et al., 2006; Yi et al., 2008).

P. alveolata has two conspicuous Ma that can easily be separated from two congeneric species: *P. lacazei* has 25-47 Ma without the an alveolar layer (*vs*. presence), whereas *P. quadrinucleata* has four Ma (*vs*. two).

Kahl (1932) described *P. alveolata* with sufficient morphological features for identification, matching it well the the present population, mainly in terms of its conspicuous alveolar layer, two Ma, three FC, two BC, and a well-developed J-shaped TC. A morphometric comparison with the recently described Chinese population is shown in Table 2. The increasing body length as the number of AZM increases is apparent along with both marginal cirri; the present population has a longer body length (149 *vs*. 96.6 μm) and a greater number of left marginal cirri (24.7 *vs*. 17) compared to the Chinese population (Song and Warren, 2000).

The sequence length of SSU rDNA in the Korean population is 1,669 bp (GeneBank Accession number GU170844) with intra-specific variation of 0.2% with the existing *P. alveolata* (DQ503583).

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